

**CLAIMS****WHAT WE CLAIM IS:**

- 5           1.       An antibody that is capable of binding CTLA-4, comprising a heavy chain variable region amino acid sequence that comprises a contiguous amino acid sequence from within an FR1 sequence through an FR3 sequence that is encoded by a human V<sub>H</sub>3-33 family gene and that comprises at least one of the amino acid substitutions in the CDR1 sequences, CDR2 sequences, or  
10       framework sequences shown in Figure 2.
2.       The antibody of Claim 1, wherein the amino acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6,  
15       SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, and SEQ ID NO:70.
3.       The antibody of Claim 1, further comprising a light chain  
20       variable region amino acid sequence comprising a sequence selected from the group consisting of a sequence comprising SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69,  
25       and SEQ ID NO:71.
4.       The antibody of Claim 2, further comprising a light chain  
variable region amino acid sequence comprising a sequence selected from the group consisting of a sequence comprising SEQ ID NO:14, SEQ ID NO:15,  
30       SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ

ID NO:25, SEQ ID NO:26, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69,  
and SEQ ID NO:71.

5           5.       An antibody comprising a heavy chain amino acid sequence  
comprising SEQ ID NO:1 and a light chain variable amino acid sequence  
comprising SEQ ID NO:14.

          6.       An antibody comprising a heavy chain amino acid sequence  
comprising SEQ ID NO:2 and a light chain variable amino acid sequence  
10       comprising SEQ ID NO:15.

          7.       An antibody comprising a heavy chain amino acid sequence  
comprising SEQ ID NO:4 and a light chain variable amino acid sequence  
comprising SEQ ID NO:17.

15           8.       A isolated human monoclonal antibody that is capable of binding  
to CTLA-4.

          9.       The antibody of Claim 8, wherein the antibody is capable of  
20       competing for binding with CTLA-4 with an antibody selected from the group  
consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1,  
11.7.1, 12.3.1.1, and 12.9.1.1.

          10.       The antibody of Claim 8, wherein the antibody possesses a  
25       substantially similar binding specificity to CTLA-4 as an antibody selected from  
the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1,  
11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1.

          11.       The antibody of Claim 8, wherein the antibody is selected from  
30       the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1,  
11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1.

12. The antibody of Claim 8, wherein the antibody is not cross reactive with CTLA-4 from lower mammalian species.

13. The antibody of Claim 12, wherein the lower mammalian species  
5 comprises mouse, rat, and rabbit.

14. The antibody of Claim 8, wherein the antibody is cross reactive with CTLA-4 from primates.

10 15. The antibody of Claim 14, wherein the primates comprise cynomolgous and rhesus monkeys.

16. The antibody of Claim 8, wherein the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than  
15 about 100:1.

17. The antibody of Claim 16, wherein the selectivity is about 500:1 or greater.

20 18. The antibody of Claim 8, wherein a binding affinity of the antibody is about  $10^{-9}$  M or greater.

19. The antibody of Claim 18, wherein a binding affinity of the antibody is about  $10^{-10}$  M or greater.

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20. The antibody of Claim 8, wherein the antibody inhibits binding between CTLA-4 and B7-2 with an  $IC_{50}$  of lower than about 100 nM.

21. The antibody of Claim 20, wherein the antibody inhibits binding  
30 between CTLA-4 and B7-2 with an  $IC_{50}$  of lower than about 0.38 nM.

22. The antibody of Claim 8, wherein the antibody inhibits binding between CTLA-4 and B7-1 with an  $IC_{50}$  of lower than about 100 nM or greater.

23. The antibody of Claim 22, wherein the antibody inhibits binding  
5 between CTLA-4 and B7-1 with an  $IC_{50}$  of lower than about 0.50 nM.

24. The antibody of Claim 8, wherein the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater.

10 25. The antibody of Claim 24, wherein the antibody enhances IL-2 production in a T cell blast/Raji assay by about 3846 pg/ml or greater.

26. The antibody of Claim 8, wherein the antibody enhances IFN- $\gamma$  production in a T cell blast/Raji assay by about 500 pg/ml or greater.

15 27. The antibody of Claim 26, wherein the antibody enhances IFN- $\gamma$  production in a T cell blast/Raji assay by about 1233 pg/ml or greater.

28. The antibody of Claim 8, wherein the antibody induces IL-2  
20 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or greater.

29. The antibody of Claim 26, wherein the antibody induces IL-2  
production in a hPBMC or whole blood superantigen assay by about 1500 pg/ml  
25 or greater

30. The antibody of Claim 26, wherein the antibody induces IL-2 production in a hPBMC or whole blood superantigen assay by greater than about 30% relative to control.

31. The antibody of Claim 26, wherein the antibody induces IL-2 production in a hPBMC or whole blood superantigen assay by greater than about 50% relative to control.

5 32. A humanized antibody that possesses a substantially similar binding specificity to CTLA-4 as an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1.

10 33. The antibody of Claim 32, wherein the antibody is not cross reactive with CTLA-4 from lower mammalian species.

34. The antibody of Claim 33, wherein the lower mammalian species comprises mouse, rat, and rabbit.

15 35. The antibody of Claim 32, wherein the antibody is cross reactive with CTLA-4 from primates.

36. The antibody of Claim 35, wherein the primates comprise  
20 cynomolgous and rhesus monkeys.

37. The antibody of Claim 32, wherein the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than about 100:1.

25 38. The antibody of Claim 37, wherein the selectivity is about 500:1 or greater.

39. The antibody of Claim 32, wherein a binding affinity of the  
30 antibody is about  $10^{-9}$  M or greater.

40. The antibody of Claim 39, wherein a binding affinity of the antibody is about  $10^{-10}$  M or greater.

41. The antibody of Claim 32, wherein the antibody inhibits binding  
5 between CTLA-4 and B7-2 with an  $IC_{50}$  of lower than about 100 nM.

42. The antibody of Claim 41, wherein the antibody inhibits binding between CTLA-4 and B7-2 with an  $IC_{50}$  of lower than about 0.38 nM.

10 43. The antibody of Claim 32, wherein the antibody inhibits binding between CTLA-4 and B7-1 with an  $IC_{50}$  of lower than about 100 nM or greater.

44. The antibody of Claim 43, wherein the antibody inhibits binding between CTLA-4 and B7-1 with an  $IC_{50}$  of lower than about 0.50 nM.

15 45. The antibody of Claim 32, wherein the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater.

46. The antibody of Claim 45, wherein the antibody enhances IL-2  
20 production in a T cell blast/Raji assay by about 3846 pg/ml or greater.

47. The antibody of Claim 32, wherein the antibody enhances IFN- $\gamma$  production in a T cell blast/Raji assay by about 500 pg/ml or greater.

25 48. The antibody of Claim 47, wherein the antibody enhances IFN- $\gamma$  production in a T cell blast/Raji assay by about 1233 pg/ml or greater.

49. The antibody of Claim 32, wherein the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml  
30 or greater.

50. The antibody of Claim 49, wherein the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by about 1500 pg/ml or greater

5 51. The antibody of Claim 32, wherein the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by greater than about 30% relative to control.

10 52. The antibody of Claim 50, wherein the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by greater than about 50% relative to control.

15 53. An antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded by a human  $V_H$  3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, the CDR1, CDR2, and CDR3 sequences being independently selected from the CDR1, CDR2, and CDR3 sequences illustrated in Figure 2.

20 54. The antibody of Claim 32, further comprising any of the somatic mutations to the FR1, FR2, and FR3 sequences as illustrated in Figure 2.

25 55. An antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded by a human  $V_H$  3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, which antibody has the following properties:  
a binding affinity for CTLA-4 of about  $10^{-9}$  or greater;  
inhibits binding between CTLA-4 and B7-1 with an  $IC_{50}$  of about 100 nM or lower;  
30 inhibits binding between CTLA-4 and B7-2 with an  $IC_{50}$  of about 100 nM or lower; and

enhances cytokine production in an assay of human T cells by 500 pg/ml or greater.

56. An antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising FR1, FR2, and FR3 sequences operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence independently selected from the CDR1, CDR2, and CDR3 sequences illustrated in Figures 2 and 3, which antibody has the following properties:

a binding affinity for CTLA-4 of about  $10^{-9}$  or greater;  
10 inhibits binding between CTLA-4 and B7-1 with an  $IC_{50}$  of about 100 nM or lower;  
inhibits binding between CTLA-4 and B7-2 with an  $IC_{50}$  of about 100 nM or lower; and  
enhances cytokine production in an assay of human T cells by  
15 500 pg/ml or greater.

57. A cell culture system for assaying T cell stimulation, comprising a culture of human T cell blasts co-cultured with a Raji cell line.

20 58. The cell culture system of Claim 57, wherein the T cell blasts are washed prior to culture with the Raji cell line.

59. An assay for measuring T cell stimulation, comprising:  
providing a culture of human T cell blasts and a Raji cell line;  
25 contacting the culture with an agent; and  
measuring cytokine production by the culture.

60. The assay of Claim 59, wherein the T cell blasts are washed prior to culture with the Raji cell line.

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61. The assay of Claim 59, wherein the cytokine is IL-2.



62. The assay of Claim 59, wherein the cytokine is IFN- $\gamma$ .

63. The assay of Claim 59, wherein cytokine production is measured in supernatant isolated from the culture.

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64. The assay of Claim 59, wherein the agent is an antibody.

65. The assay of Claim 59, wherein the antibody binds to CTLA-4.

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66. A functional assay for screening a moiety for T cell stimulatory function, comprising:

providing a culture of human T cell blasts and a Raji cell line;  
contacting the culture with the moiety; and  
assessing cytokine production by the culture.

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67. The assay of Claim 66, wherein the T cell blasts are washed prior to culture with the Raji cell line.

68. The assay of Claim 66, wherein the cytokine is IL-2.

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69. The assay of Claim 66, wherein the cytokine is IFN- $\gamma$ .

70. The assay of Claim 66, wherein cytokine production is assessed in supernatant isolated from the culture.

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71. The assay of Claim 66, wherein the moiety is an antibody.

72. The assay of Claim 66, wherein the antibody binds to CTLA-4.

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73. A T cell stimulatory assay for CTLA-4 inhibitory function, comprising contacting a culture comprising human T cell blasts and a Raji cell line with an agent and assessing cytokine production by the culture.

74. The assay of Claim 73, wherein the T cell blasts are washed prior to culture with the Raji cell line.

5 75. The assay of Claim 73, wherein the cytokine is IL-2.

76. The assay of Claim 73, wherein the cytokine is IFN- $\gamma$ .

77. The assay of Claim 73, wherein cytokine production is assessed  
10 in supernatant isolated from the culture.

78. The assay of Claim 73, wherein the agent is an antibody.

79. The assay of Claim 73, wherein the antibody binds to CTLA-4.

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80. A method for screening an agent for T cell stimulatory activity, comprising:

contacting the agent with a cell culture comprising human T cell blasts and a Raji cell line; and

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assessing cytokine production by the culture.

81. The method of Claim 80, wherein the T cell blasts are washed prior to culture with the Raji cell line.

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82. The method of Claim 80, wherein the cytokine is IL-2.

83. The method of Claim 80, wherein the cytokine is IFN- $\gamma$ .

84. The method of Claim 80, wherein cytokine production is  
30 assessed in supernatant isolated from the culture.

85. The method of Claim 80, wherein the agent is an antibody that binds to CTLA-4.

86. An assay for measuring T cell stimulation, comprising:  
5 providing a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A;  
contacting the culture with an agent; and  
measuring cytokine production by the cell population.

10 87. The assay of Claim 86, wherein the cytokine is IL-2.

88. The assay of Claim 86, wherein cytokine production is measured in supernatant isolated from the cell population.

15 89. The assay of Claim 86, wherein the agent is an antibody.

90. The assay of Claim 86, wherein the antibody binds to CTLA-4.

20 91. A functional assay for screening a moiety for T cell stimulatory function, comprising:  
providing a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A;

25 contacting the culture with the moiety; and  
assessing cytokine production by the cell population.

92. The assay of Claim 91, wherein the cytokine is IL-2.

30 93. The assay of Claim 91, wherein cytokine production is assessed in supernatant isolated from the cell population.

94. The assay of Claim 91, wherein the moiety is an antibody .
95. The assay of Claim 91, wherein the antibody binds to CTLA-4.
- 5 96. A T cell stimulatory assay for CTLA-4 inhibitory function, comprising contacting a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A with an agent and assessing cytokine production by the cell population.
- 10 97. The assay of Claim 96, wherein the cytokine is IL-2.
98. The assay of Claim 96, wherein cytokine production is assessed in supernatant isolated from the cell population.
- 15 99. The assay of Claim 96, wherein the agent is an antibody.
100. The assay of Claim 96, wherein the antibody binds to CTLA-4.
101. A method for screening an agent for T cell stimulatory activity, comprising:  
20 contacting the agent with a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A; and  
assessing cytokine production by the cell population.
- 25 102. The method of Claim 101, wherein the cytokine is IL-2.
103. The method of Claim 101, wherein cytokine production is assessed in supernatant isolated from the cell population.
- 30 104. The method of Claim 101, wherein the agent is an antibody that binds to CTLA-4.